EXPERIMENTAL STUDIES WITH ENDAMOEBA GROS*

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It is but a short time since, that investigators[1] announced the cause of pyorrhea alveolaris as an entameba found in the pus around the root of the affected tooth. This assertion was unaccompanied by any experimental evidence. To conclude that an entameba found in the pus around the root of a tooth is responsible for the production of pyorrhea alveolaris is no more rational than to conclude that any other of the many different species of bacteria found there is responsible.

The technic pursued in the experimental study of Endamoeba Gros reported here, was as follows:

The cheesy debris covering the neck of the affected tooth is removed with a pledget of cotton held by thumb forceps. Immediately the platinum loop is sterilized in a gas or alcohol flame, and after cooling is carefully passed to the bottom of the root socket. Care must be exercised in passing the loop into the root socket for the reason that there is a marked hypervascularization of the tissues in the immediate vicinity of the root of the tooth; slight trauma causes a hemorrhage which at times is difficult to control, and the loop, which should contain a sample of pus, contains erythrocytes instead. The loop having been passed without accident into the deepest portion of the root socket of the diseased tooth, is moved upward, downward, and laterally, the object being to collect a specimen from the entire area of disease. Then the loop is carefully withdrawn from the root socket, and its content mixed with a drop of sterile salt solution on a clean slide. A cover slip is placed over the drop of emulsion and firmly pressed against the slide. The specimen is now ready for microscopic examination. Having determined the presence of active entamebas, we are ready to commence the actual isolation of the protozoa.

The technic for the preparation of the cell, and of the cover slip, the making of the capillary pipets, the centering of the pipets, and the operation of the pipet holder has been described.[2] The preparation of cell and cover slip having been completed, a coarse capillary pipet is made, to the free end of which is fastened a piece of rubber tubing. The free end of the tubing is placed between the lips, and the point of the pipet is immersed in sterile broth in a test tube; suction by the mouth then draws any desired quantity of broth into the pipet. With the pipet charged and carefully passed under the cover slip, a series of small droplets is deposited on the under surface of the cover slip by making slight pressure with the mouth on the rubber hose. On the completion of this step another specimen of pus is collected from around

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[1] Barrett and Smith: Dent. Cosmos, 1914, 56, p. 948. Bass and Johns: Jour. Am. Med. Assn., 1915, 64, p. 554.

[2] Hecker: Jour. Infect. Dis., 1916, 19, p. 306.

the root of the tooth as before. An emulsion of this specimen is made in the small droplets farthest from the outer edge of the cover slip. The cell is now made secure in the mechanical stage. Fine capillary pipets, to the free ends of which are fastened pieces of rubber tubing, are then charged with sterile broth and centered.

The droplets are now searched for active entamebas, and as soon as one is found, Pipet 1 is gradually moved upward until its point is faintly in focus. The cell is then moved by the mechanical stage until the protozoan is directly over the point of the pipet. Then the latter is moved upward until it comes in contact with the entameba, and the instant that it does the protozoan commences to enter. If at this time gentle suction is made with the mouth, the entameba enters the pipet very quickly. The instant that it enters the pipet, the pipet is immediately moved out of focus. We have now picked up the protozoan, but it is not yet free from all other bacteria. After the entameba has been isolated, the cell is moved to the left until a droplet of the water of condensation is found on the surface of the cover slip. The pipet is then moved upward until the point appears in this droplet. Gentle pressure is made with the mouth until the protozoan appears in the droplet and then is instantly stopped, the pipet being at the same time moved out of focus. This procedure is repeated until one has isolated the desired number of entamebas. The pipets used for this step are now removed from the pipet holder to be replaced with new pipets, which are smaller in caliber; the rubber tubes are attached to them, and they are charged with sterile broth and centered.

One of the small droplets containing an entameba and bacteria is now located. Pipet 1 is moved upward until the point is faintly in focus. The bacteria in the droplet are then collected in the pipet. As soon as all have been removed, the pipet is moved downward out of focus. As the droplet containing the entameba and the bacteria is, many times, markedly depleted in its liquor content by the removal of the bacteria, the size of the droplet is restored by moving Pipet 2 upward until the point of the pipet comes in contact with the droplet and making pressure with the mouth on the rubber hose until the desired quantity of liquor has been added. Each of the droplets containing an isolated entameba and bacteria is subjected to the foregoing technic.

The bacteria having been removed from the droplets containing the isolated entameba, the next step is that of removing the entamebas from the droplets with Pipet 2 and placing each of them in a new droplet. Then, after all the isolated entamebas have been transferred with Pipet 2 to new droplets, the pipets are removed from the holder. Two new pipets are now made, which are much larger in caliber than the ones employed for the isolation of the bacteria; the rubber hose is attached to them, and they are charged with sterile broth, and centered.

Each of the isolated protozoa is picked up with Pipet 1 and washed from 3 to 5 times in the sterile broth contained in the pipet. This rids their surfaces of any debris or bacteria which would make the experiment faulty. After each entameba has been washed, it is picked up with Pipet 2 and placed in a drop along the line on the cover slip which is at an angle to the two lines at right angles to each other.

Again the pipets are removed from the holder and one of large caliber is made, and the rubber hose attached to it. It is then charged with sterile broth, and a line is made with india ink on the shank of the pipet as a guide to the quantity of liquor it contains; it is then centered. The drop is now sought which contains the washed isolated entamebas, and they are collected

in the pipet one at a time. As soon as all of them have been collected, the pipet is moved downward out of focus. The preparation of the animal for inoculation is then commenced.

Guinea-pigs of standard size are used. The animal, wrapped in a large towel, its head exposed, is placed on its back in the lap and grasped gently by the thighs to prevent its moving. The pipet is removed from the holder and the shank of the pipet grasped by the thumb and index and second fingers of the left hand. With the left hand the rubber hose is placed between the lips. Traction is then made on the lip of the guinea-pig until the gingiva is freely exposed, whereupon the point of the pipet is carefully passed between the gum and the root of the tooth until the deepest portion of the gingiva is reached. The instant that this point is reached gentle pressure is made with the mouth on the free end of the rubber hose held by the lips, the liquor in the pipet gradually passes into the gingival space, and as soon as it reaches the guide line on the shank of the pipet, the pressure is stopped and the pipet immediately withdrawn. The guinea-pig is laid aside for 1 hour. At the end of this time it is unwrapped and placed in its pen, the date, the point of inoculation, and the dose having been noted.

The gingival space of the left and right upper and lower central incisors, meso-approximal and distal surfaces, were thus injected in 6 guinea-pigs. The number of organisms inoculated ranged from 5 to 18. Careful macroscopic and microscopic examinations at the point of inoculation were made daily, but, with the exception of 3 guinea-pigs which showed slight inflammation of the gingivae on the 2nd and 3rd days respectively, none of the animals disclosed inflammation, pus, or presence of entamebas during a period of 84 days following the inoculation.

The author also injected active entamebas into the gingival space around the teeth in his own mouth. Careful microscopic examination was made of the contents of the gingival space of each of the 10 teeth selected for inoculation, and in no instance did the author find entamebas. The teeth selected were the 6 lower anterior teeth and the 4 anterior upper teeth.

The entamebas were isolated from an emulsion of pus from around the root of the affected tooth in the same manner as described in connection with the animal experiments. The difficult step was the development of a technic for the inoculation of the gingival space by the author himself, unassisted. The following method became easy after a little practice.

The pipet employed is the same in type as that used in the animal experiments. The entameba having been picked up, the pipet is lowered out of focus. The gum overlying the site selected for inoculation is then carefully massaged, and the neck of the tooth is wiped to free the gingival margin of all debris. The pipet is then removed from the pipet holder, and the shank grasped by

the thumb and index finger of the right hand, while the left holds the rubber bulb of an ear syringe, which is attached to the free end of the rubber tubing attached to the pipet. The worker, seated in front of a mirror, gently passes the point of the pipet into the deepest portion of the gingival space, until the point meets with resistance. Gentle pressure with the left hand on the bulb of the ear syringe forces the liquor slowly from the pipet into the gingival space; as soon as the liquor reaches the guide line on the shank of the pipet, the pressure is stopped and the pipet is instantly withdrawn. The inoculated area is given a liberal coating of the compound tincture of benzoin, which is allowed to harden. The traction on the lip is stopped, and the lip allowed to return to its normal position.

The largest number of active entamebas thus injected into the gingival space was 14. Macroscopic examinations were made of the area of inoculation daily for 7 days following. After the 7th day microscopic examinations were made. The first experiment was commenced on March 12, 1916, and the succeeding experiments followed at 5-day intervals. The areas of inoculation were carefully observed daily until August 1, 1916, and up to this time no inflammation of the gum had occurred, and no pus, and no entamebas had been found microscopically.

From the foregoing experiments we learn that the gingival space of the guinea-pig and the healthy gingiva of man, when inoculated with active entamebas, do not develop inflammation or pus and that the entamebas do not survive to reproduce their species. Hence, at this time, we can not accept the theory that this protozoan, when found in the pus around the root of a tooth affected by pyorrhea alveolaris, is etiologically responsible for the production of the malady.